

REMARKS

1. Introduction

1.1. We have revised the 119(e)/120 reference as requested by the Examiner. We wish to note that the language used was customary at the time of the May 17, 2002 amendment.

1.2. We have corrected the misspellings "naphtalene" and "dichlorophenoxyactic acid" in the specification and in claim 12. We respectfully suggest that the term "naptalam" is not misspelled.

1.3. The Examiner is thanked for allowing claims 1-10, 14-22, 27, 28, 31, 32, 38 and 39.

2. Definiteness Issues

2.1. We have corrected the Markush group terminology in claim 12. See comments in 1.2 above.

2.2. We have amended claim 36 to depend from claim 33.

3. Prior Art Issues

The only claims rejected over prior art are claims 33 and 36, both rejected as anticipated by and/or obvious over Becwar et al.

Claim 33 has been amended to recite that the embryo is "non-desiccated". The basis for this amendment is at page 15, lines 31-35. Since this recites that a dessication step "may" be "advantageous", the implication is that a dessication step may be omitted, resulting in a non-desiccated embryo.

Claim 36 is now dependent on claim 33.

Embryos can be produced by a plant in nature by fertilization of the egg cell by the sperm cell of the pollen. These embryos are denoted zygotic (sexual) embryos and are a part of the seed. In *in vitro* cultures embryos can be produced from somatic tissue and without a fertilization step, and these embryos are denoted somatic embryos.

Conifers belong to the plant group Gymnosperm. Generally, the production process of somatic embryos for gymnosperms can be

presented as a process including the following steps:

1. Initiation of embryogenic cultures: Establishing of embryogenic cultures from somatic cells.
2. Maintenance (or proliferation) of embryonal suspensor masses: Continued production of new embryos in the embryogenic cultures. By cleavage of existing embryos or from single cells.
3. Maturation: Development of embryos into mature embryos.
4. Germination and conversion to plants.

Each of these steps may comprise culturing on one or more media. Said media typically include elements to induce or maintain the developmental stage of interest.

The conifer somatic embryos produced by the prior art methods have a poorer capability for germination and further development than zygotic embryos of conifers. Somatic embryos produced by prior art methods contain less dry matter (stored nutritions) but much more water than zygotic embryos, thus these somatic embryos have a high water content, which is correlated with a low germination success.

In the prior art documents the germination of the somatic embryos has been increased by drying of the somatic embryos. Thus the prior art methods initially produce non-desiccated somatic embryos with a high water content and then the water content of the somatic embryos are lowered by the desiccation step.

With the present invention the germination of the somatic embryos has been increased by producing somatic embryos which by themselves (i.e., without a special drying step) have the water content lower than 70%.

The somatic embryos produced by the method in the present application have a water content less than 70% when they are mature and not exposed to a drying step. This is obtained by culturing the somatic embryos by the maturation method according

to the present invention comprising a step with an anti-auxin within the culture media. Thus the water content below 70% of the somatic embryos produced by the present invention is obtained without performing any drying method.

US 5,413,930

US 5,413,930 of Becwar et al describes a method for regeneration of coniferous plants by somatic embryogenesis by a multi-step method. The multi-step method includes a maturation drying treatment also denoted a 'partial drying' of the embryos by exposing the embryos to an atmosphere having a high relative humidity for sufficient time to permit the embryos to lose about 25% to 75% of their pre-dried weight, this technique were applied to the somatic embryos as a preparation for germination.

The multi-step method of Becwar et al. includes all the steps 1 to 4 of the list presented above, as well as the partial drying treatment of the somatic embryos which is performed between step 3 and step 4.

The present invention describes a method for maturation of conifer somatic embryos, that is step 3 of the list presented above, by culturing an embryogenic cell mass with a culture medium comprising an anti-auxin.

The low water content of the somatic embryos of Becwar et al. is obtained in the **desiccated** somatic embryos, whereas the low water content of the somatic embryos of the present invention is obtained in **non-desiccated** somatic embryos.

Becwar et al. does not describe maturation of conifer somatic embryos by culturing with a culture medium comprising an anti-auxin in step 3 of the list mentioned above. Nor does it describe the water content of the somatic embryos before desiccation that is of the non-desiccated somatic embryos.

In Becwar et al. neither the water content before the "partial drying" nor followed the "partial drying" is presented, thus there is no indication to what the water content of the mature somatic embryos are **before the desiccation**.

Becwar et al. does therefore not show that the mature non-desiccated somatic embryos have a water content less than 70%, as there is no indication of the water content of the somatic embryos before or after drying, and it is not possible to calculate any specific water content of the non-desiccated somatic embryos.

In Becwar et al. the mature somatic embryos are desiccated to increase germination of the somatic embryos. Such a dessication process is not included in the method of the present invention, and claim 33 has been amended to specify this.

The drying of Becwar's somatic embryo by external methods (e.g. heat) can be expected to result in morphological and metabolic changes that distinguish it from Applicant's somatic embryo, which is innately of low water content. Thus claim 33 distinguishes Becwar et al.

Respectfully submitted,

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